Amino acid positron emission tomography to monitor chemotherapy response and predict seizure control and progression-free survival in WHO grade II gliomas

Roelcke, U ; Wyss, M T ; Nowosielski, M ; Rudà, R ; Roth, P ; Hofer, S ; Galldiks, N ; Crippa, F ; Weller, M ; Soffietti, R

Abstract: BACKGROUND Patients with WHO grade II glioma may respond to chemotherapy that is currently not standardized regarding timing and treatment duration. Metabolic changes during chemotherapy may precede structural tumor volume reductions. We therefore compared time courses of amino acid PET and MRI responses to temozolomide (TMZ) and assessed whether responses correlated with seizure control and progression-free survival (PFS). METHODS PET and MRI were performed before and during TMZ chemotherapy. Tumor volumes were calculated using regions-of-interest analysis. Amino acid uptake was also quantified as metabolically active tumor volume and tumor-to-cerebellum uptake ratio. RESULTS One hundred twenty-five PET and 125 MRI scans from 33 patients were analyzed. Twenty-five patients showed metabolic responses that exhibited an exponential time course with a 25% reduction of the active volume on average after 2.3 months. MRI responses followed a linear course with a 25% reduction after 16.8 months. Reduction of metabolically active tumor volumes, but not reduction of PET uptake ratios or MRI tumor volumes, correlated with improved seizure control following chemotherapy (P = .012). Receiver-operating-characteristic curve analysis showed that a decrease of the active tumor volume of 80.5% predicts a PFS of 60 months (P = .018) and a decrease of 64.5% a PFS of 48 months (P = .037). CONCLUSIONS Amino acid PET is superior to MRI for evaluating TMZ responses in WHO grade II glioma patients. The response delay between both imaging modalities favors amino acid PET for individually tailoring the duration of chemotherapy. Additional studies should investigate whether this personalized approach is appropriate with regard to outcome.

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Amino acid PET to monitor chemotherapy response, and to predict seizure control and progression-free survival in WHO grade II gliomas

Ulrich Roelcke MD[1*], Matthias T. Wyss MD, PhD[2,3*], Martha Nowosielski MD, PhD[4], Roberta Rudà MD[5], Patrick Roth MD[6], Silvia Hofer MD[7], Norbert Galldiks MD[8], Flavio Crippa MD[9], Michael Weller MD[6], Riccardo Soffietti MD[5].

[1]Department of Neurology and Brain Tumor Center, Cantonal Hospital, 5001 Aarau, Switzerland; [2]Institute for Pharmacology and Toxicology and [3]Neuroscience Center, ETH and University of Zürich, 8091 Zürich, Switzerland; [4]Department of Neurology, Medical University, 6020 Innsbruck, Austria; [5]Department of Neuro-Oncology, University Hospital, 10126 Torino, Italy; Departments of [6]Neurology and [7]Oncology, University Hospital, 8091 Zürich, Switzerland; [8]Department of Neurology, University Hospital, 50924 Cologne, and Research Center, 52425 Jülich, Germany; [9]Medicina Nucleare, Istituto Nazionale dei Tumori, 20133 Milano, Italy.

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Correspondence:

Prof. Dr. med. Ulrich Roelcke

Department of Neurology & Brain Tumor Center

Cantonal Hospital, Aarau

5001 Aarau, Switzerland

Tel +41 62 838 56 82

Fax +41 62 838 98 58

Email roelcke@ksa.ch

*These authors contributed equally to this study.

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Abstract

Background. Patients with WHO grade II glioma may respond to chemotherapy which is currently not standardized regarding timing and treatment duration. Metabolic changes during chemotherapy may precede structural tumor volume reductions. We therefore compared time courses of amino acid PET and MRI responses to temozolomide (TMZ), and assessed whether responses correlate with seizure control and progression-free survival (PFS).

Methods. PET and MRI were performed before and during TMZ chemotherapy. Tumor volumes were calculated using regions-of-interest analysis. Amino acid uptake was also quantified as metabolically active tumor volume and tumor:cerebellum uptake ratio.

Results: 125 PET and 125 MRI scans from 33 patients were analyzed. Twenty-five patients showed metabolic responses which exhibited an exponential time course with a 25% reduction of the active volume on average after 2.3 months. MRI responses followed a linear course with a 25% reduction after 16.8 months. Reduction of metabolically active tumor volumes, but not reduction of PET uptake ratios or MRI tumor volumes, correlated with improved seizure control following chemotherapy ($p = 0.012$). Receiver-operating-characteristic curve analysis showed that a decrease of the active tumor volume of ≥80.5% predicts a PFS of ≥60 months ($p = 0.018$), and of ≥64.5% a PFS of ≥48 months ($p = 0.037$).

Conclusions. Amino acid PET is superior to MRI to evaluate TMZ responses in WHO grade II glioma patients. The response delay between both imaging modalities favours amino acid PET to individually tailor chemotherapy duration. Additional studies should investigate whether this personalized approach is appropriate with regard to outcome.

Key words: low-grade glioma – chemotherapy – PET – MRI - epilepsy

Introduction

Diffuse cerebral WHO grade II gliomas often present with epileptic seizures and show insidious tumor growth.[1] The natural history and response to treatment of these tumors is predominantly determined by genetic alterations such as co-deletion on chromosomal arms 1p and 19q, isocitrate dehydrogenase 1 (IDH1/2) mutation and methylation of the *O*^{6}-*methylguanine-DNA methyltransferase (MGMT)* promoter.[2,3]

Alkylating agent chemotherapy is active in patients with WHO grade II glioma. Once given at tumor progression or recurrence it yields objective response rates up to 60% and response durations of several years.[4] In addition, chemotherapy can reduce the frequency of seizures. However, the evaluation of chemotherapy response based on MRI with T_2-weighted and FLAIR MRI sequences is difficult.[5] Accordingly, a reduction in seizure frequency has been proposed as a surrogate marker for a clinical benefit from chemotherapy.[6,7] Currently there is no standard for the optimal drug choice, timing and duration of chemotherapy. The European Organization for Research and Treatment of Cancer trial 22033-26033 prescribed 12 cycles of temozolomide (TMZ) chemotherapy, while other groups treated patients as long as MRI showed at least evidence of stable disease.[4] Interestingly, tumors may continue to shrink on MRI even after termination of chemotherapy.[8,9] Using PET with the amino acid *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) we reported rapid metabolic deactivation in WHO grade II glioma patients in response to TMZ.[10] Our current study aimed to investigate metabolic responses in a large multicenter patient cohort treated with TMZ. We also assessed whether metabolic responses on PET correlate with seizure control and progression-free survival (PFS).

Materials and Methods

Clinical data. Patients were retrospectively identified according to the following criteria: (i) supratentorial cerebral WHO grade II glioma at progression after surgery; (ii) no contrast enhancement on T_1-weighted MRI; (iii) no previous radio- or chemotherapy; (iv) measurable disease on T_2-weighted MRI before chemotherapy with two perpendicular diameters ≥ 10 mm; (v) a MRI and amino acid PET schedule including a baseline evaluation and at least two examinations during and at the end of chemotherapy. Patient data were extracted from the hospital medical records and included demographic as well as tumor and treatment characteristics, PFS and overall survival (OS). Seizure frequencies before chemotherapy were documented during post-surgical surveillance in three-monthly intervals. During chemotherapy seizure frequencies were recorded monthly. We defined seizure control groups during chemotherapy as: group I = 0-50% seizure frequency reduction, group II = >50% reduction or seizure-free.

Chemotherapy. TMZ chemotherapy was administered according to local policies (Table 1). Adverse effects were scored according to the Common Terminology Criteria for Adverse Events (CTCAE, version 4.0).

Imaging data acquisition. Baseline imaging was performed within one month before chemotherapy initiation, and follow-up imaging started between 2 and 6 months thereafter. MRI examinations included T_2-weighted and gadolinium-enhanced T_1-weighted sequences according to clinical routine protocols on 1.5 or 3.0 Tesla MRI scanners. PET data were acquired after intra-venous administration of 150-240 MBq FET (Switzerland, Austria, Germany) or approximately 650 MBq L-[methyl-11C]methionine (MET, Italy). Grosu et al reported that both tracers yield virtually identical amino acid uptake values in gliomas.[11] Their whole cohort of gliomas WHO II-IV showed mean tumor:brain uptake ratios of 2.5 for FET and of 2.6 for MET which represents a difference of 4%. For the subgroup of WHO II gliomas uptake ratios differed by 1%.[11] We therefore consider both MET and FET suitable to

comparably characterize low-grade gliomas on amino acid PET. Static emission scans were acquired at the following time periods after tracer administration: 40-60 min (Switzerland; Discovery LS, GE Medical Systems), 30-45 min (Austria; Advance, GE Healthcare), 20-40 min (Germany; ECAT Exact HR+, Siemens), 30-45 min (Italy; Discovery LS, GE Healthcare). After correction for random and scattered coincidences and dead time, images were reconstructed with the specific scanner software. The reconstructed image resolution was approximately 5.5 mm.

Image data analysis, determination of response and progression. Response assessment on PET and MRI was centrally performed by M.Wy. (Nuclear Medicine) and U.R. and N.G. (Neurooncologists) with long-standing experience in PET and MRI analysis. Each patient served as his own control, and values during chemotherapy were expressed as percent from baseline, i.e. before the start of chemotherapy. Quantification of PET and MRI data was performed on co-registered images using PMOD (PMOD Technologies Ltd, Zurich, Switzerland).[12] MRI tumor volumes were measured on T_2-weighted axial sequences by manually outlining regions-of-interest (ROIs) around tissue exhibiting T_2-hyperintensity. Finally, all tumor-containing slices were summed. Values during chemotherapy were expressed as percent change from baseline MRI before initiation of chemotherapy. Response was also determined according to the Response Assessment in Neuro-Oncology (RANO) criteria which quantify the product of the perpendicular tumor diameters on the slice showing the largest tumor area (mm^2).[5] The RANO criteria categorize size reductions between 25% and 50% as ‚minor response', ≥50% as 'partial response', and disappearance of the lesion on T_2–weighted or FLAIR MR imaging as 'complete response'. 'Objective response' is the sum of minor response, partial response and complete response. Size increases of ≥25% are scored as ‚progressive disease'. 'Stable disease' is defined when MRI changes do not qualify for progression, or for complete, partial, or minor response. We diagnosed progression also if

new contrast enhancement on T_1-weighted sequences or clinical signs (e.g. recurrence or new onset of seizures) were accompanied by tumor size increase less than 25%.

Amino acid uptake was also quantified using ROI analysis. The cerebellum served as reference region. ROIs covering the whole cerebellum were drawn for each slice. Counts of all cerebellar ROIs were then averaged to produce the mean cerebellar amino acid uptake. For tumors three measures were used: First, the 'metabolically active volume' (cm^3) was calculated as the tumor volume containing pixels >110% of the mean cerebellar amino acid uptake. We derived this threshold from our earlier methodological study.[13] It corresponds well to the visual impression of active WHO grade II gliomas on PET.[14] Second, the tumor amino acid uptake (counts) was determined by placing a ROI over the tumor on the PET slice with the largest extent and which was normalized to the mean cerebellar uptake (mean T:CBL ratio, unit-less). Third, peak uptake ratios were calculated by averaging all counts from tumor voxels which exhibited ≥95% of the maximum tumor radioactivity. In patients without active tumor volume, ROIs were placed on the T_2-weighted MR images, and were then transferred to the corresponding PET planes. In line with our previous study[10] we defined a metabolic treatment response as a reduction of 10% or more from the initial tumor volume (active PET volume) on at least two subsequent time points at least 4 weeks apart after the initiation of chemotherapy.

Molecular genetics. 1p/19q co-deletion was assessed by fluorescence *in situ* hybridization, and MGMT promoter methylation by methylation-specific PCR. IDH1 status was determined by immunohistochemistry or gene sequencing.

Statistics. We used descriptive statistics to characterize the patient population. To test group-to-group differences the Mann Whitney U test for independent samples was applied. The association between baseline imaging parameters and PFS was assessed using the Spearman Rank test. The prognostic value of the metabolically active tumor volume on PFS was

assessed by receiver-operating-characteristic (ROC) curve analyses. Patients were divided into responders (PFS ≥12, 24, 36, 48, 60 months) and non-responders (PFS <12, 24, 36, 48, 60 months). Decision cut-off was considered optimal when the product of paired values for sensitivity and specificity reached its maximum. Moreover, we determined the area under the ROC curve (AUC), its standard error and level of significance as measures of diagnostic quality. In addition, we used uni- and multivariate analysis including logistic regression to test for associations of histology, PET and MRI response, total dose of TMZ, as well as 1p/19q co-deletion, IDH1 and MGMT status with PFS. PFS and OS were calculated from the start of chemotherapy. Analyses were performed using SigmaStat software (version 3.5; Systat Software, Inc.).

Ethics. Amino acid PET examinations are part of routine clinical investigations in WHO grade II glioma patients in Austria, Italy, Switzerland and Germany. All patients gave written informed consent before each FET or MET PET investigation. Signed consent is not required to perform clinical PET scans in Switzerland. The local ethics committee of Innsbruck Medical University approved this retrospective study. In Italy local ethics committee approval or patient's signed consent is not required for retrospective data evaluation.

Results

Thirty-three patients with WHO grade II astrocytoma (n=6), oligodendroglioma (n=20) or oligoastrocytoma (n=7) were enrolled (21 male, 12 female, mean age 37 ± 8 (SD) years, range 20 - 53). Prior to chemotherapy 29 patients underwent one, and 4 patients two surgical procedures (gross total resection in 6, partial resection in 18, biopsy in 9 patients, Table 1). During the whole study no patient received steroids. No patient was treated with either previous radiotherapy or PCV (procarbazine-lomustine-vincristine) chemotherapy.

Chemotherapy. TMZ chemotherapy started 40 ± 36 months (range 1-126) after the last operation because of radiological progression. Chemotherapy was completed as scheduled in 17 patients, and was interrupted because of patient's request, toxicity or progression in 1, 7 and 8 patients, respectively. Hematological adverse effects of CTCAE grades 1, 2 or 3 were noted in 5, 3 and 8 patients, respectively. Non-hematological adverse effects consisted of nausea in 8 (grades 1-3), fatigue in 6 (grades 2-3), anorexia in 2 (grades 1-2), pruritus in 2 (grade 2), and local skin infection/zoster in 2 patients (grades 2-3). No secondary neoplasias were observed after a median follow up of 74 months.

Imaging. All patients from Italy were imaged with MET-PET (n=9). Patients from all other countries were imaged with FET-PET (n=24). 125 PET and 125 MRI scans each were available for evaluation. On MRI 14 patients achieved an objective response (9 minor responses, 5 partial responses) (Table 1). Baseline tumor volumes on T_2-weighted MRI ranged from 4.3 - 450.3 cm^3 (75.7 ± 103.9 cm^3). Among those 30 patients with baseline active tumor defined by PET, volumes ranged from 1.4 - 269.9 cm^3 (51.2 ± 51.2 cm^3). In line with previous work[11] we found similar baseline mean tumor uptake ratios for MET (1.43 ± 0.27) and FET (1.42 ± 0.20). For histological subtypes the following numbers were found: T:CBL ratios were 1.43 ± 0.24 in oligodendrogliomas, 1.43 ± 0.27 in oligoastrocytomas, and 1.28 ± 0.14 in astrocytomas. Peak T:CBL ratios were 2.34 ± 2.29 in oligodendrogliomas, 2.49 ± 1.99 in oligoastrocytomas, and 1.80 ± 1.58 in astrocytomas. In 3 patients we found no active tumor volume at baseline. In these patients the median T:CBL ratio was 1.04 ± 0.02. We identified 25 metabolic responders and 5 non-responders. Examples of individual PET and MRI responses are presented in Figure 1A. Metabolic responses during chemotherapy were best described by an exponential time course, which yielded a reduction of 25% after 2.3 months (Figure 1B). In contrast, MRI responses on T_2-weighted images of the same patients were delayed, followed a linear decrease and showed a volume reduction of 25%

16.8 months after initiation of chemotherapy (Figure 1C). During chemotherapy the mean and peak T:CBL ratios in PET responders were reduced in seven patients by 10-21%. The remaining patients showed decreases of less than 7%. This most likely reflects that successful chemotherapy reduces the spatial extent of tumor burden, i.e. the active tumor volume, but not histology or WHO tumor grade. In contrast to the responses of the active tumor volume T:CBL ratios did not follow a linear or an exponential fit (data not shown).

In PET non-responding patients active tumor volumes increased within six months up to 354% from baseline. The mean T:CBL ratios increased by 7-11%, and the peak T:CBL ratios by 14-36%. All tumors without active volume remained metabolically inactive during chemotherapy, and T:CBL ratios remained in a range between 96 and 103% from baseline.

Molecular genetic data. Information on IDH1 status and LOH1p/19q was available in 26 patients each, and on MGMT promoter methylation in 24 patients (Table 1). 22 (85%) tumors showed IDH1 mutation, 13 (50%) 1p/19q co-deletion, and 20 (83%) MGMT promoter methylation. PET responders exhibited IDH1 mutation in 17 (77%) patients, 1p19q co-deleted tumors in 11 (85%) patients, and MGMT promoter methylation in 17 (85%) patients.

Seizures. Twenty-two patients presented with seizures before chemotherapy. Tumor size on MRI, active PET tumor volume and tumor uptake ratios at baseline were balanced between the 2 seizure response groups (Table 2). During chemotherapy antiepileptic drug doses were changed to account for low drug serum concentration or for persisting seizures in 5 patients, respectively, and were stable in 17 patients. During chemotherapy no patient experienced an increase in seizure frequency. Nine patients experienced seizure frequency reductions by 0-50%, 6 patients by >50%, and 7 patients were seizure-free. Among the 17 patients under stable antiepileptic drug doses TMZ reduced the active tumor volumes by 22 ± 27% in group I patients, and by 64 ± 28% in group II patients (Figure 2A, $p = 0.012$, Mann Whitney test).

In contrast, neither T:CBL ratio changes nor volume changes on T_2-weighted MRI images corresponded with seizure frequency reductions (Figure 2B,C).

Survival. At the time of last follow-up 25 patients were alive, 7 patients were dead, and one patient was lost to follow-up. Twenty-six patients progressed at 33 ± 22 months (range, 6 - 85 months) after the start of chemotherapy, while 7 patients were free from progression at 42 ± 22 months (6 - 68). Progression was characterized by neurological deterioration in 23, by increase of the T_2 lesion in 24, and by appearance of new contrast enhancement in 14 patients, respectively. Nine patients underwent re-operation for progressive tumor (Table 1). Of the progressing patients 7 died 45 ± 19 months (21 - 75) after start of chemotherapy. Neither the mean nor the peak T:CBL ratios, nor the magnitude of active tumor volumes prior to chemotherapy were predictive for PFS ($p = 0.551$, $p = 0.748$ and $p = 0.622$, respectively). In patients with active tumor volume (n = 30) the median PFS was not significantly different between metabolic responders (34.3 ± 21.8 months, range 6 - 85) and non-responders (27.6 ± 27.2 months, range 6 - 67) ($p = 0.605$). In contrast, ROC analysis yielded a decrease of the active tumor volume of ≥80.5% as an optimal cut-off for the prognostication of a PFS of ≥60 months (sensitivity 67%, specificity 90%, accuracy 83%, AUC 0.78 ± 0.02; $p = 0.018$). For the prognostication of a PFS of ≥48 months, a decrease of the active tumor volume of ≥64.5% was the optimal threshold (sensitivity 70%, specificity 58%, accuracy 62%, AUC 0.74 ± 0.11; $p = 0.037$). The prognostication of a PFS of ≥36 months ($p = 0.154$), ≥24 months ($p = 0.369$), and ≥12 months ($p = 0.057$), respectively, was not significant.

The PFS in patients without active tumor volume was 29.1 ± 12.4 months (17 - 41). None of the following parameters correlated with PFS, either on univariate or multivariate analysis: histology (oligodendroglial/oligoastrocytic versus astrocytic tumor, $p = 0.988$), PET (responder versus non-responder, $p = 0.734$), MRI (objective response (RANO), $p = 0.276$),

total TMZ dose (p = 0.100), IDH1 status (p = 0.088), 1p/19q co-deletion (p = 0.331), MGMT promoter methylation status (p = 0.672) (p values shown for univariate analysis). In addition, also group-to-group comparisons (imaging responses) did not show differences between tumors with or without IDH mutation, between tumors with or without co-deletion 1p19q, or between tumors with methylated or unmethylated MGMT promotor (Mann Whitney U test, p>0.5 for all comparisons). This may – in part – be explained by the small subgroups compared with this test.

Discussion

In line with previous reports on patients with diffuse cerebral WHO grade II gliomas we have observed that chemotherapy responses on MRI occur with variable delay from the initiation of treatment.[8,9] We noticed a 25% tumor volume reduction on MRI after 16.8 months, whereas a 25% reduction of the metabolically active tumor volume on PET was observed as early as 2.3 months after initiation of chemotherapy. We excluded patients with enhancing tumors on MRI because BBB disruption contributes to non-specific imaging results through passive radiotracer influx into the tumor. Thus, we can assume that the amino acid PET signal in our patients is specific for active amino acid uptake[15,16] which for MET and FET is largely mediated by the L-amino acid transport system.[17] Accordingly, TMZ chemotherapy appears to down-regulate active amino acid transport in WHO grade II gliomas as an early response indicator. Altogether, 83% of our patients with active tumor volume were PET responders following exposure to TMZ. The long delay between PET and MRI responses may reflect that amino acid transporters represent a sensitive nutritional part of tumor cell metabolism, whereas signal abnormalities on MRI T_2-weighted sequences which may relate to tumor cell densities, edema, and alteration of the extracellular matrix respond slowly to cytotoxic therapy.

Apart from tumor control, TMZ chemotherapy resulted in improved seizure control. At best of our knowledge this is the first study showing that seizure control corresponds to the magnitude of active tumor volume reductions on amino acid PET. Of note, seizure control was neither paralleled by changes in amino acid uptake ratios, nor by decreases of tumor volumes as measured by MRI. The latter is in line with recent observations in patients with WHO grade II gliomas treated with either radiotherapy[18] or TMZ.[7] Thus, improved seizure control may not rely exclusively on reduced compression of neuronal structures by the tumor but also on the reduction of magnitude and spatial extent of local metabolic disequilibrium. The mechanisms behind improved seizure control may involve the glutamatergic system. Gliomas release glutamate as an excitatory neurotransmitter and activate neuronal glutamate receptors.[19,20] FET uptake is increased during status epilepticus in the cortex adjacent to gliomas and resolves after cessation of epileptic activity.[21] Whether transport of both types of amino acids, which under physiological conditions use different transport systems, relates to each other in gliomas remains to be evaluated.

The main limitation of our study is its retrospective nature and small sample size. Moreover, there was heterogeneity of TMZ regimens and PET and MRI scanners, and molecular data were not available for all patients. The variability of TMZ regimens reflects the current lack of evidence-based recommendations regarding the optimal regimen and duration of chemotherapy.[22] However, based on our results, we assume that the time delay between amino acid PET and MRI response may be clinically meaningful. Individual PET-guided determination of chemotherapy duration in patients showing an early metabolic response and response plateau could reduce the risk of toxicity, and lower treatment burden and costs. Moreover, one should be cautious to overtreat patients with alkylating chemotherapy which can exert a mutagenic effect influencing the risk of malignant transformation.[23,24] In conclusion, we here characterize the patterns of chemotherapy response on amino acid PET

in a cohort of patients with diffuse cerebral WHO grade II gliomas, and report a positive correlation between active tumor volume responses, PFS and seizure control. A prospective study should validate our findings including MRI, dynamic amino acid PET[25] and molecular markers to determine whether personalized PET-guided management has impact on survival. With regard to the choice of amino acid PET tracer FET seems currently to be most promising.[26] For the purpose of chemotherapy response assessment FET uptake should be quantified as metabolically active volume. This may represent a useful approach to patients with WHO grade II gliomas, a rare disease with substantial variation of the clinical course and treatment response.

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Figure Captions

Figure 1. Time course of imaging responses

A: Examples of individual responses as measured with PET (filled symbols) and MRI (open symbols). Square: oligoastrocytoma (#12); triangle: oligodendroglioma (#16); diamond: astrocytoma (#6). B, C: Data points represent pooled volume changes measured for the whole cohort of metabolic responders during chemotherapy before progression, that is, patients were censored at the last PET or MRI prior to progression: black lines correspond to exponential fit for PET data (B) ($y = 74.61 * \exp(-x/158.86) + 24.31$) and linear fit for MRI data (C) ($y = 99.27 – 0.05 * x$), respectively. Values on the y-axis represent percent volume changes compared to baseline, i.e. before the start of chemotherapy.

Figure 2. Seizure control and imaging responses

Best imaging responses on active PET volume (A), mean T:CBL uptake ratio (B), MRI T_2 lesion size (C) in patients under stable antiepileptic drug doses (n = 17). Seizure control group I = reduction of seizure frequency by 0-50%, seizure control group II = reduction >50% or seizure-free patients during chemotherapy. Figure 2A: *p = 0.012 (Mann Whitney U test).